

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-78 (Canceled).

Claim 79 (Currently Amended). A tandem fluorescent protein ~~construct~~
construct comprising:

i) a donor fluorescent protein moiety comprising ~~[[an]]~~ **a contiguous** amino acid sequence **of 150 amino acids having at least 85% sequence identity with a sequence of substantially identical to** SEQ ID NO:2, and which differs from SEQ ID NO:2 **by at least a set of** amino acid substitutions selected from the group **of sets** consisting of:

- a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys;
- b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr;
- c) Tyr66His and Tyr145Phe;
- d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;
- e) Ser72Ala, Tyr145Phe and Thr203Ile; and
- f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr;

characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore; and

ii) an acceptor fluorescent protein moiety comprising ~~[[an]]~~ **a contiguous** amino acid sequence **of 150 amino acids having at least 85% sequence identity with a sequence of substantially identical to** SEQ ID NO:2, and which differs from SEQ ID NO:2 by **at least a set of** amino acid substitutions selected from the group **of sets** consisting of:

- a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr; and

b) Ser65Thr, ~~Ser72Ala~~ Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr; characterized in that amino acid residues in said acceptor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, and

iii) a linker moiety that couples the donor moiety of i) and the acceptor moiety of ii), wherein the linker moiety comprises a protease recognition site;

wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited; and

wherein the donor moiety acceptor moiety and the linker moiety are fused in a single amino acid sequence.

Claim 80 (Currently Amended). A tandem fluorescent protein construct comprising:

i) a donor fluorescent protein moiety comprising ~~[[an]]~~ a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least a set of amino acid substitutions selected from the group of sets consisting of:

a) Tyr66His and Tyr145Phe; and

b) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;

characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore,

ii) an acceptor fluorescent protein moiety comprising ~~[[an]]~~ a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least an amino acid substitution~~[[s]]~~selected from the group consisting of:

a) Ser65Cys; and

b) Ser65Thr,

characterized in that amino acid residues in said acceptor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, and

iii) a linker moiety that couples the donor moiety of i) and the acceptor moiety of ii), wherein the linker moiety comprises a protease recognition site;

wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited; and

wherein the donor moiety acceptor moiety and the linker moiety are fused in a single amino acid sequence.

Claim 81 (Currently Amended). A tandem fluorescent protein construct comprising:

A) a donor fluorescent protein moiety comprising:

i) **[[an]] a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of substantially identical to SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least a set of amino acid substitutions selected from the group of sets consisting of:**

a) Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163A and Asn212Lys;

b) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr;

c) Tyr66His and Tyr145Phe;

d) Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala and Asn212Lys;

e) Ser72Ala, Tyr145Phe and Thr203Ile; and

f) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr;

characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, or

ii) **[[an]] a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with a sequence of ~~substantially identical to~~ SEQ ID NO:2 and comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223, wherein the mutation attenuates the intermolecular interactions between the donor and acceptor moieties, characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore;**

B) an acceptor fluorescent protein moiety comprising:

i) **[[an]] a contiguous amino acid sequence of 150 amino acids having at least 85% sequence identity with the sequence of ~~substantially identical to~~ SEQ ID NO:2, and which differs from SEQ ID NO:2 by at least a set of amino acid substitutions selected from the group of sets consisting of:**

a) Ser65Gly, Val68Leu, Ser72Ala and Thr203Tyr; and

b) Ser65Thr, Ser72Ala, Asn149Lys, Met153Thr and Ile167Thr; or

ii) **[[an]] a contiguous amino acid sequence having at least 85% sequence identity with a sequence of ~~substantially identical to~~ SEQ ID NO:2 and comprising a mutation that reduces the hydrophobicity at positions A206, L221 or F223, wherein the mutation attenuates the intermolecular interactions between the donor and acceptor moieties, characterized in that amino acid residues in said donor fluorescent protein moiety corresponding to positions 65 to 67 of SEQ ID NO:2 are oxidized and cyclized to form a fluorophore, and**

C) a linker moiety that couples the donor moiety of A) and the acceptor moiety of B), wherein the linker moiety comprises a protease recognition site;

wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited; and

wherein the donor moiety acceptor moiety and the linker moiety are fused in a single amino acid sequence.

Claim 82 (New). The construct of claim 79, wherein the donor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with the sequence of SEQ ID NO:2 and the acceptor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with a sequence of SEQ ID NO:2.

Claim 83 (New). The construct of claim 80, wherein the donor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with SEQ ID NO:2 and the acceptor fluorescent protein moiety comprises a contiguous amino acid sequence having at least 95% sequence identity with a sequence of SEQ ID NO:2.

Claim 84 (New). The construct of claim 81, wherein the donor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with the sequence of SEQ ID NO:2 and the acceptor fluorescent protein moiety comprises a contiguous amino acid sequence of 200 amino acids having at least 95% sequence identity with a sequence of SEQ ID NO:2.

Claim 85 (New). The construct of claim 79, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

Claim 86 (New). The construct of claim 80, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

Claim 87 (New). The construct of claim 81, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

Claim 88 (New). The construct of claim 82, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

Claim 89 (New). The construct of claim 83, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

Claim 90 (New). The construct of claim 84, wherein the linker moiety comprises between 5 amino acids and 50 amino acids.

Claim 91 (New). The construct of claim 79, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 92 (New). The construct of claim 80, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 93 (New). The construct of claim 81, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 94 (New). The construct of claim 82, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 95 (New). The construct of claim 83, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 96 (New). The construct of claim 84, wherein the linker comprises a cleavage recognition site for trypsin, enterokinase, HIV -1 protease, prohormone convertase, interleukin-1b-converting enzyme, adenovirus endopeptidase, cytomegalovirus assemblin, leishmanolysin, β -secretase for APP, thrombin, renin, angiotensin-converting enzyme, cathepsin D or a kininogenase.

Claim 97 (New). The construct of claim 79, wherein the donor fluorescent protein fluorescent protein moiety has at least 85% sequence identity with the sequence of SEQ ID NO:2 and the acceptor fluorescent protein fluorescent protein moiety has at least 85% sequence identity with the sequence of SEQ ID NO:2.

Claim 98 (New). The construct of claim 82, wherein the donor fluorescent protein fluorescent protein moiety has at least 95% sequence identity with the sequence of SEQ ID NO:2 and the acceptor fluorescent protein fluorescent protein moiety has at least 95% sequence identity with the sequence of SEQ ID NO:2.

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PATENT

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Claim 99 (New). The construct of claim 98, wherein the linker is polypeptide from 12 to 40 amino acids in length